Osmotic Stress Induces Expression of Choline Monooxygenase in Sugar Beet and Amaranth

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Choline monooxygenase (CMO) catalyzes the committing step in the synthesis of glycine betaine, an osmoprotectant accumulated by many plants in response to salinity and drought. To investigate how these stresses affect CMO expression, a spinach (Spinacia oleracea L., Chenopodiaceae) probe was used to isolate CMO cDNAs from sugar beet (Beta vulgaris L., Chenopodiaceae), a salt- and drought-tolerant crop. The deduced beet CMO amino acid sequence comprised a transit peptide and a 381-residue mature peptide that was 84% identical (97% similar) to that of spinach and that showed the same consensus motif for coordinating a Rieske-type [2Fe-2S] cluster. A mononuclear Fe-binding motif was also present. When water was withheld, leaf relative water content declined to 59% and the levels of CMO mRNA, protein, and enzyme activity rose 3- to 5-fold; rewatering reversed these changes. After gradual salinization (NaCl:CaCl$_2$ = 5:1, mol/mol), CMO mRNA, protein, and enzyme levels in leaves increased 3- to 7-fold at 400 mM salt, and returned to uninduced levels when salt was removed. Beet roots also expressed CMO, most strongly when salinized. Salt-inducible CMO mRNA, protein, and enzyme activity were readily detected in leaves of Amaranthus caudatus L. (Amaranthaceae). These data show that CMO most probably has a mononuclear Fe center, is inducibly expressed in roots as well as in leaves of Chenopodiaceae, and is not unique to this family.

Like other organisms, many plants accumulate betaines, polyols, or Pro in response to dry or saline conditions (Yancey, 1994; Bohnert et al., 1995). These compounds, termed compatible solutes or osmoprotectants, serve as nontoxic solutes for cytoplasmic osmoregulation and can also partly reverse the damaging effects of salts on proteins and membranes (Yancey, 1994). The metabolic engineering of osmoprotectant accumulation has therefore attracted interest as a way to improve crop stress resistance (McCue and Hanson, 1990; Bartels and Nelson, 1994; Bohnert and Jensen, 1996). One target for such engineering is Gly betaine, a potenti osmoprotectant that occurs widely among flowering plants, including the economically important families Chenopodiaceae, Amaranthaceae, Malvaceae, Compositae, and Gramineae (Gorham, 1995).

Plants synthesize Gly betaine via a two-step oxidation of choline: choline $\rightarrow$ betaine aldehyde $\rightarrow$ Gly betaine (Rhodes and Hanson, 1993). Bacteria use the same route, which has led several groups to express bacterial genes for choline oxidation (choline dehydrogenase or choline oxidase) in plants that lack Gly betaine, in some cases targeting the enzyme to chloroplasts because this is the site of Gly betaine synthesis in Chenopodiaceae (Rhodes and Hanson, 1993). The transformants made small amounts of Gly betaine and were significantly more salt tolerant (e.g. Lilius et al., 1996; Hayashi et al., 1997).

It is now possible to use plant genes for such engineering experiments, since cDNAs for both enzymes of Gly betaine synthesis have been cloned from Chenopodiaceae (McCue and Hanson, 1992a; Rathinasabapathi et al., 1997). The enzyme mediating the second reaction is BADH, an NAD-linked dehydrogenase that is known also from Amaranthaceae and Gramineae (Ishitani et al., 1993; Valenzuela-Soto and Munoz-Clares, 1994). In Chenopodiaceae, BADH is predominantly (≥ 90%) located in the chloroplast stroma (Rathinasabapathi et al., 1994). Salinity and water deficit raise BADH mRNA and enzyme levels in leaves and roots, coincident with the accumulation of Gly betaine (McCue and Hanson, 1992a; Ishitani et al., 1995; Wood et al., 1996). BADH cDNAs from Chenopodiaceae and Gramineae have recently been functionally expressed in tobacco (Rathinasabapathi et al., 1994; Ishitani et al., 1995).

The enzyme catalyzing the first and committing step of Gly betaine synthesis is not as well known, having so far been found only in spinach (Chenopodiaceae). The spinach enzyme (CMO) is a stromal, Fd-dependent monooxygenase with a Rieske-type [2Fe-2S] center, and it is completely unrelated to the bacterial choline dehydrogenase and oxidase enzymes (Burnet et al., 1995; Rathinasabapathi et al., 1997). Little is known about CMO expression except that it increases in salinized spinach leaves (Brouquisse et al., 1989; Rathinasabapathi et al., 1997).

We cannot yet answer three questions about the natural expression of CMO that bear on its use in metabolic engineering. First, is it induced by drought as well as by salinity? Second, is it expressed in roots as well as in leaves? Third, is it unique to Chenopodiaceae or is it found in other Gly betaine-accumulating plants? To address the first two questions we chose sugar beet (Chenopodiaceae) because it

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Abbreviations: BADH, betaine aldehyde dehydrogenase; CMO, choline monooxygenase; $\Psi_s$, solute potential; RWC, relative water content.
can accumulate high levels of Gly betaine in both roots and shoots (≃ 300 μmol g⁻¹ dry weight), it is a halophyte, and it withstands drought well (Hanson and Wyse, 1982; Durham, 1993). This led us to isolate beet CMO cDNAs for use as homologous probes; analysis of their deduced amino acid sequences revealed a mononuclear Fe-binding motif.

MATERIALS AND METHODS

Plants and Growing Conditions

Sugar beet (Beta vulgaris L., cv Great Western D-2) seed was obtained from Hilleshög Mono-hy, Inc. (Longmont, CO). Beet plants from which mRNA was isolated for library construction were grown in a growth chamber (12-h day, 25°C, 350 μE m⁻² s⁻¹ PPFD/12-h night, 22°C) and salinized with 400 mM NaCl as described previously (McCue and Hanson, 1992a). For salinity experiments, beet plants were grown hydroponically (four per 18-L tank) in aerated one-half-strength Hoagland nutrient solution in a growth chamber (8-h day, 25°C, 350 μE m⁻² s⁻¹ PPFD/16-h night, 20°C). Salinization began when plants were 4 weeks old, using an NaCl:CaCl₂ mixture (5.7:1 mol/mol, hereafter termed salt), and the concentration was raised by 50 mM every third day to final values of 100, 250, or 400 mM salt. Water was added daily to replace evapotranspiration and the medium was replaced every 2 weeks. Plants were harvested 3 d after the final concentration was reached in the 400 mM salt treatment, collecting four young leaves (blade length about 5–20 cm) and the taproot from each. At the same time, one batch of four plants was transferred from 250 mM salt to nutrient solution alone, and harvested 3 d later. Unsalinized controls were harvested at the same time as the 400 mM salt treatment or at a similar stage of development to the plants in this treatment; since the data were similar, only the latter are presented.

For drought experiments, beet plants were grown (one per 4-L pot) in heavy potting soil (Southland Importers, Greensboro, NC) supplemented with 5 g per pot of Osmocote 14:14:14 (N:P:K) (Scotts-Sierra, Maysville, OH) in a greenhouse in natural daylight during November and December, 1996; minimum temperature was 18°C. Irrigation was with one-half-strength Hoagland nutrient solution. Seven weeks after planting, irrigation was withheld for 7 d, by which time the expanded leaves had been wilted continuously for 3 d. One-half of the plants was then harvested, taking young leaves as above; the others were rewatered and harvested 3 d later. Control leaves were harvested just before irrigation was withheld. In all experiments, samples from individual plants were frozen in liquid N₂ and stored at −80°C.

Amaranth (Amaranthus caudatus L. cv RRC 1036) seeds were obtained from U.S. Department of Agriculture, North Central Regional Plant Introduction Station (Ames, IA). Plants were grown (two per 2.5-L pot) in Metro-Mix 300 (Grace Sierra, Milpitas, CA), supplemented with Osmocote, in the 8-h day/16-h night conditions given above. Salinization was with NaCl:CaCl₂ in nutrient solution as above, starting at 5 weeks, applying 1.5 L per pot daily, and raising the salt level by 50 mM every third day to a final value of 300 mM. Controls were irrigated with nutrient solution. After 3 d at 300 mM salt, young, fully expanded, and expanding leaves (≃ 50% final size) were harvested, frozen, and stored as above.

Measurement of Ψₛ and RWC

The fourth youngest leaf was sampled for beet, and the first fully expanded leaf was sampled for amaranth. Ψₛ was determined on frozen-thawed 0.8-cm diameter leaf discs using a thermocouple psychrometer (HR-33T, Wescor, Logan, UT) equipped with C-52 sample chambers. RWC was measured on sets of nine 1.6-cm discs from the same leaves, as described by Rhodes et al. (1987).

Gly Betaine Analysis

Gly betaine was extracted from 50-mg dry weight leaf samples with a methanol/chloroform/water procedure and isolated by ion-exchange chromatography (Hanson et al., 1991). [Methyl-²H₃]Gly betaine (448 nmol/sample) was added at the start of the extraction as internal standard. Gly betaine was converted to its n-butyl ester and determined by fast atom bombardment MS as described by Rhodes et al. (1987).

RNA Isolation and Analysis

For beet cDNA library construction, total RNA was isolated as described previously (Rathinasabapathi et al., 1997) and poly(A⁺) RNA was prepared using poly(U) Sephadex (Hondred et al., 1987). For expression studies, total RNA was isolated by a single-step method (Puissant and Houdebine, 1990). Poly(A⁺) RNA for expression studies was purified using polyA Spin Kits (New England Biolabs). RNA was separated on 1.2% formaldehyde gels and blotted to Duralon membranes (Stratagene); molecular size standards (0.24- to 9.5-kb RNA ladder, Gibco-BRL) were included. Probes were labeled (> 10⁶ cpm μg⁻¹) by the random primer method using [α-³²P]dCTP. Autoradiographs of northern blots were scanned using a digital imaging system (IS-1000, Alpha Innotech, San Leandro, CA) to quantify signals. Signal strengths were normalized with respect to the amount of RNA in each track, determined by reprobing blots with an 18S rRNA probe (Nairn and Ferl, 1988). The quality of amaranth poly(A⁺) RNA preparations was checked by probing blots with the 1.6-kb XhoI fragment of amaranth PEP carboxylase (Rydzik and Berry, 1996).

cDNA Cloning and DNA Sequence Analysis

A cDNA library (3.1 × 10⁶ plaque-forming units) was constructed in the UniZap XR vector (Stratagene). Screening and in vivo excision were carried out according to the supplier’s protocols. Filters were hybridized at 42°C and washed at 55°C in 2× SSC containing 0.1% (v/v) SDS. The amplified library was screened with a 1150-bp AccI-EcoRV fragment of spinach CMO cDNA clone pRS3 (Rathinasabapathi et al., 1997) labeled as described above. This
yielded a near-full-length CMO cDNA (SB2), of which the 5'-terminal 265-bp EcoRI fragment was used to isolate clone SB30 from the unamplified library. Clones were sequenced in both strands using the fluorescent chain-terminating dideoxynucleotides method. Sequences were analyzed using the Wisconsin GCG Sequence Analysis Package (Version 8.0, Genetics Computer Group, Madison, WI).

**Protein Isolation and Analysis**

Samples (2 g fresh weight) were pulverized in liquid N₂ and extracted with 8 mL of ice-cold buffer containing 100 mm Tris, 1 mm Na₂EDTA, 10 mm DTT, and 4% (w/v) PVP, adjusted to pH 8.0 with HCl; for all samples except the ascorbic acid, 1 m Na₂EDTA, 0.5 m PMSF, 1 μg mL⁻¹ leupeptin, and 1 μg mL⁻¹ antipain were also included. After centrifuging at 12,000 g (10 min, 4°C), proteins were precipitated from the supernatant by adding 1 volume of ice-cold 50% (w/v) PEG 8000 in 25 mm Tris-HCl, pH 8.0 (without or with borate, ascorbate, leupeptin, and antipain as above), holding on ice for 2 h, and centrifuging at 9,000 g (15 min, 4°C). This procedure was shown to precipitate 80% of the protein present in beet leaf extracts. The pellet was redissolved in a buffer (adjusted to pH 8.0 with HCl) containing Tris 50 mm, 0.5 mm Na₂EDTA, 2 mm DTT, 10% (v/v) glycerol, and, except for drought-stressed beet leaves, 1 μg mL⁻¹ leupeptin and 1 μg mL⁻¹ antipain. The solution was then desalted on Sephadex G-25 equilibrated in the same buffer and used for CMO assays and western analyses. CMO activity was measured by the coupled radiometric assay described previously (Burnet et al., 1995) using an incubation time of 10 or 20 min, and adjusting the amount of extract so that product formation was proportional to time and protein concentration. Proteins were separated by SDS-PAGE and transferred to nitrocellulose as described previously (Tohoku University of Agriculture, 1985). Blots were probed with mouse polyclonal antibodies to spinach CMO (Rathinasabapathi et al., 1997).

**RESULTS**

**Isolation and Characterization of Beet CMO cDNAs**

A cDNA library was prepared using mRNA from salt-stressed beet leaves, and screened with a spinach CMO cDNA (Rathinasabapathi et al., 1997). This yielded a 5'-truncated CMO clone, SB2 (1709 bp). A 5' fragment of SB2 was then used to isolate SB30, a 3'-truncated clone that included 36 bp of 5'-untranslated region and 468 bp of coding sequence, of which 462 bp overlapped with SB2. Together, SB2 and SB30 represent a full-length cDNA of 1751 bp that encodes a polypeptide of 446 amino acids and has a 377-bp 3'-untranslated region. The composite amino acid sequence is shown in Figure 1, aligned with that of spinach CMO. Comparison of the beet and spinach N-terminal regions suggests that beet CMO has a 65-residue stromal targeting peptide, similar in length to that of spinach CMO (60 residues) and of typical amino acid composition (Cline and Henry, 1996). The deduced amino acid sequence of the mature beet CMO polypeptide has 84% identity and 97% similarity to that of spinach, which are comparable values to those for their BADHs (McCue and Hanson, 1992a). Beet CMO contains the consensus sequence (Cys-X-His-X15–17-Cys-X2-His) for coordinating a Rieske-type [2Fe-2S] cluster-binding motif, as found in spinach CMO (Rathinasabapathi et al., 1997). Solid arrows mark the conserved Cys-His pairs of the Rieske-type [2Fe-2S] cluster-binding region, and open arrows indicate the conserved residues of the mononuclear Fe-binding motif. The accession number for the composite beet CMO nucleotide sequence is AF 023132.

**CMO Expression in Beet Leaves during and after Water Deficit**

Prolonged withholding of irrigation resulted in marked declines in RWC and Ψₛ, which were fully reversed upon...
rewatering (Table I). As expected, this drought treatment promoted Gly betaine accumulation, although it did not lead to measurable overall osmotic adjustment (i.e. a net increase in solutes), since \( \Psi_w \) values corrected to 100% RWC did not fall. Consistent with the accumulation of Gly betaine, during the stress period the level of CMO mRNA rose 5-fold (Fig. 2A), CMO protein accumulated (Fig. 2B), and CMO enzyme activity tripled (Fig. 2C). Within 3 d of rewatering, CMO mRNA, protein, and enzyme activity had all fallen to or below their prestress levels (Fig. 2). The size of the CMO mRNA observed in all treatments (1.9 kb) was slightly above that of spinach (1.7 kb; Rathinasabapathi et al., 1997).

**CMO Expression in Salinized Beet Leaves and Roots**

Adding salt to the hydroponic medium lowered its \( \Psi_w \) by about 0.55 MPa at 100 mM and 1.9 MPa at 400 mM, as predicted (Wyn Jones and Gorham, 1983). The declines in leaf \( \Psi_w \) in salinized plants were close to these values, and resulted from both osmotic adjustment and, to a lesser extent, a drop in RWC (Table II). In leaves the steady-state levels of CMO mRNA, protein, and enzyme activity all rose as salinity was increased, and at 400 mM salt they were 4- to 7-fold higher than in unsalinized controls (Fig. 3). Three days after relief of salt stress, RWC and \( \Psi_w \) had both risen markedly (Table II), and CMO mRNA, protein, and enzyme activity had fallen back to levels no higher than those in control leaves (Fig. 3).

In taproots CMO mRNA levels were undetectable in the control and low in the 100 mM salt treatment, but at 400 mM salt they were comparable to those in leaves (Fig. 4A). CMO protein level and activity (Fig. 4, B and C) were also far higher at 400 mM salt, but were readily detectable in both other treatments. Expressed per unit protein, CMO activities in roots were higher than in leaves (Figs. 3C and 4C). SDS-PAGE analysis of the PEG-precipitated fractions used to assay CMO indicated that this reflected the high proportion of Rubisco in leaf samples.

**Table I. Responses of sugar beet leaves to a cycle of drought stress and relief**

Irrigation was withheld for 7 d, by which time the mature leaves had been wilted for 3 d, and then restored for 3 d. Control plants were irrigated daily. The \( \Psi_w \) and RWC measurements were made between 10:00 AM and 1:00 PM; data are means of four replicates and were subjected to analysis of variance. The \( \Psi_w \) values shown are not corrected to 100% RWC. Gly betaine was determined using leaf discs pooled from four plants and is expressed on the basis of fresh weight at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RWC*</th>
<th>( -\Psi_w^* )</th>
<th>Gly Betaine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>MPa</td>
<td>( \mu mol g^{-1} ) fresh wt</td>
</tr>
<tr>
<td>Control</td>
<td>91.1a</td>
<td>1.14a</td>
<td>11.8</td>
</tr>
<tr>
<td>Drought stress</td>
<td>58.5b</td>
<td>1.69b</td>
<td>23.1</td>
</tr>
<tr>
<td>Drought/rewater</td>
<td>89.4a</td>
<td>1.12a</td>
<td>17.0</td>
</tr>
</tbody>
</table>

* Means within columns not followed by the same letter are significantly different at the 5% probability level.

**Figure 2. Expression of CMO in sugar beet leaves during a cycle of drought stress and relief.** Irrigation was withheld until mature leaves had been continuously wilted for 3 d (Drought), and then restored for 3 d (Rewater). Controls were irrigated daily. A, Northern analysis. Lanes contained 10 \( \mu \)g of total RNA; the blot was probed with two EcoRI fragments comprising the 5'-terminal 815 bp from beet clone SB2. The numbers beneath each lane show the CMO mRNA abundance relative to the control (1.0), normalized for loading using 18S rRNA as a benchmark. B, Western analysis. Lanes contained 130 \( \mu \)g of protein (precipitated with 25% PEG); the blot was probed with mouse antibodies raised against spinach CMO. C, CMO activity in 25% PEG-precipitated protein (means and SE for three replicates). The experiment was repeated with similar results.

**CMO Expression in Amaranth**

The families Amaranthaceae and Chenopodiaceae belong to the order Caryophyllales and are considered to stand close to each other within it (Takhtajan, 1969). Consistent with such a relationship, the beet CMO cDNA hybridized to a salt-inducible 1.9-kb CMO mRNA from amaranth leaves (Fig. 5A), and antibodies to spinach CMO

**Table II. RWC and \( \Psi_w \) of leaves from salinized beet plants**

Plants were salinized gradually and then maintained at the various salinity levels for at least 3 d. One batch of plants was salinized to a salt level of 250 mM, then transferred to nutrient solution for 3 d before harvest. Controls received nutrient solution alone. The \( \Psi_w \) and RWC measurements were made 3 to 6 h after the start of the light period; \( \Psi_w \) values were not corrected to 100% RWC. Data are means of four or five replicates and were subjected to analysis of variance.

<table>
<thead>
<tr>
<th>Salt Treatment</th>
<th>RWC*</th>
<th>( -\Psi_w^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>MPa</td>
</tr>
<tr>
<td>Control</td>
<td>93.5a</td>
<td>0.70a</td>
</tr>
<tr>
<td>100 mM</td>
<td>78.4b</td>
<td>1.20b</td>
</tr>
<tr>
<td>400 mM</td>
<td>73.4c</td>
<td>2.36c</td>
</tr>
<tr>
<td>250 mM/0 mM</td>
<td>85.6d</td>
<td>1.08b</td>
</tr>
</tbody>
</table>

* Means within columns not followed by the same letter are significantly different at the 5% probability level.
recognized an amaranth CMO polypeptide (Fig. 5B) of the same mass (about 45 kD) as the CMO monomer from spinach and beet. CMO enzyme activity was also found in control and salinized amaranth leaves (Fig. 5C), at levels comparable to those in leaves of beet (Fig. 3C) and spinach (Rathinasabapathi et al., 1997).

**DISCUSSION**

We have isolated and characterized cDNAs encoding CMO from a second plant species, with spinach being the first. The deduced amino sequence of beet CMO contained the Rieske-type [2Fe-2S] cluster-binding motif found in the spinach sequence (Rathinasabapathi et al., 1997), as well as a newly recognized consensus motif for a mononuclear Fe-binding site (Jiang et al., 1996; Gray et al., 1997). The presence of a mononuclear Fe center in CMO fits with findings for other oxygenases. Thus, various bacterial oxygenases with Rieske [2Fe-2S] clusters are known also to have mononuclear Fe centers (whence the consensus motif) that are thought to be the site of dioxygen activation and catalysis (Mason and Cammack, 1992; Jiang et al., 1996).

If CMO contains mononuclear Fe, this could help to explain why CMO loses activity during purification (Burernet et al., 1995), since mononuclear Fe is essential for catalysis but is sometimes readily lost (Suen and Gibson, 1993; Jiang et al., 1996). Consistent with this possibility, purified CMO had an Fe:S ratio of 1.06:1 (Rathinasabapathi et al., 1997), well below the expected value of 1.5:1 for a protein with a Rieske-type [2Fe-2S] cluster and a mononuclear Fe center. A systematic study of the effects of Fe²⁺ treatment on the activity of purified CMO would consequently be of interest, although it could be complicated by the effect of Fe²⁺ on superoxide-driven Fenton chemistry (hydroxyl radical formation) in the CMO assay itself (Asada, 1996).

Subjecting beet to a cycle of water stress and relief showed that drought leads to Gly betaine accumulation in leaves and that this is associated with up-regulated CMO gene expression manifest in higher levels of CMO mRNA, protein, and enzyme activity. The latter increases were completely reversed within 3 d of stress relief, indicating that both CMO mRNA and protein can be turned over quite rapidly. In this experiment, as well as in those involving salinity, the increases and decreases in CMO protein as judged from western blots were roughly comparable to those in enzyme activity. This suggests that stress does not affect CMO activity by posttranslational mechanisms, which fits with mass spectral evidence against CMO having covalent posttranslational modifications (Rathinasabapathi et al., 1997).

**Figure 3.** CMO expression in sugar beet leaves after long-term salinization. Plants were salinized gradually and then maintained at the salinity levels shown for at least 3 d. One batch of plants (250/0) was salinized to an intermediate salt level (250 mM), then transferred to nutrient solution for 3 d before harvest; a prior experiment had demonstrated that CMO induction at 250 mM salt was the same as at 400 mM. Controls received nutrient solution alone. A, Northern analysis; numbers below the lanes show the CMO mRNA abundance relative to the unsalinized control (1.0), normalized for RNA loading. B, Western analysis; lanes contained 50 µg of protein. C, CMO activity (means and se for three replicates). Other experimental conditions were as in Figure 2. The experiment was repeated with similar results.

**Figure 4.** CMO expression in sugar beet taproots after long-term salinization. The plants were the same as those used for the leaf data of Figure 3. A, Northern analysis; numbers beneath the lanes show the CMO mRNA abundance relative to that in unsalinized leaves (1.0; Fig. 3), normalized for RNA loading. B, Western analysis; lanes contained 50 µg of protein. C, CMO activity (means and se for three replicates). Other experimental conditions were as in Figure 2. The experiment was repeated with similar results.
out outside the family Chenopodiaceae. However, since the
Amaranthaceae are phylogenetically close to the Chenopo-
diaceae, it would be premature to assume that CMO also
occurs in more distant families such as Malvaceae, Com-
positae, and Gramineae. No work has yet been done on the
biochemistry of choline oxidation in these groups, but it
was recently reported that BADH in Gramineae may be
located mainly in peroxisomes rather than chloroplasts
(Nakamura et al., 1997). If Gly betaine synthesis in Gra-
mineae occurs in the peroxisome, then CMO is unlikely to
be the choline-oxidizing enzyme, because its Fd require-
ment would not be met in this compartment. It may also be
significant that we have been unable to detect CMO mRNA
or activity in cotton (Malvaceae), although reprobing the
RNA blots with a cotton α-tubulin cDNA gave satisfactory
signals, and when cotton and beet tissues were mixed and
extracted together, the beet CMO was not inactivated (B.L.
Russell and A.D. Hanson, unpublished data).

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LITERATURE CITED

Asada K (1996) Radical production and scavenging in the chloro-
123–150

Bartels D, Nelson D (1994) Approaches to improve stress toler-

Bohnert HJ, Jensen RG (1996) Strategies for engineering water-
stress tolerance in plants. Trends Biotechnol 14: 89–97

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to envi-
ronmental stresses. Plant Cell 7: 1099–1101

Reductant for glutamate synthase is generated by the oxidative
pentose phosphate pathway in non-photosynthetic root plas-
alds. Plant J 2: 893–898

Bowsher CG, Huckleby DP, Emes MJ (1989) Nitrite reduction and
carbohydrate metabolism in plastids purified from roots of
Psun sativum L. Planta 177: 359–366

Brouquisse R, Weigel P, Rhodes D, Yocum CF, Hanson AD
(1989) Evidence for a ferredoxin-dependent choline monooxy-
genase from spinach chloroplast stroma. Plant Physiol 90:
322–329

Burnet M, Lafontaine PJ, Hanson AD (1995) Assay, purification,
and partial characterization of choline monooxygenase from

Cline K, Henry R (1996) Import and routing of nucleus-encoded

279–309

Gorham J (1995) Betaines in higher plants: biosynthesis and role in
stress metabolism. In RM Wallsgrove, ed, Amino Acids and
Their Derivatives in Higher Plants. Cambridge University Press,
Cambridge, UK, pp 173–203

of cell death in plants encoded by the Lls1 gene of maize. Cell 89:
25–31
Choline Monooxygenase Expression 865


Hondred D, Wadle D-M, Titus DE, Becker WM (1987) Light-


McCue KF, Hanson AD (1992a) Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. Plant Mol Biol 18: 1–11


Rathinasabapathi B, McCue KF, Gage DA, Hanson AD (1994) Metabolic engineering of glycine betaine synthesis: plant betaine aldehyde dehydrogenases lacking typical transit peptides are targeted to tobacco chloroplasts where they confer betaine aldehyde resistance. Planta 193: 155–162


